Environmental Health Perspectives
Vol. 99, pp. 221–223, 1993

A Nonlinear Dosimetric Model for Hemoglobin Adduct Formation by the Neurotoxic Agent Acrylamide and Its Genotoxic Metabolite Glycidamide

by Carl Johan Calleman,[1] Emma Bergmark,[1] Louis G. Stern,[2] and Lucio G. Costa[1]

Hemoglobin (Hb) adducts, formed by the neurotoxic agent acrylamide (AA) and its genotoxic metabolite glycidamide (GA), were measured in the rat by means of a method for simultaneous determination of the adducts formed to cysteine. A novel, nonlinear dosimetric model was developed to describe Hb adduct formation. This model incorporates the saturable kinetics of the metabolic conversion *in vivo* of AA to GA. The pharmacokinetic parameters V_{max} and K_m and the first-order rates of elimination, k_1 and k_2, for AA and GA from all processes except conversion of AA to GA, were estimated directly from Hb adduct data to 19 M hr^{-1}, 66 μM, 0.21 hr^{-1}, and 0.48 hr^{-1}, respectively. At low concentrations, approximately 60% of AA was metabolized to GA. The nonlinear dosimetric model for adduct formation has potential general applicability in high-to-low-dose extrapolation of genotoxic effects.

The development of analytical methods for quantitating adducts formed by electrophilic substances with macromolecules such as hemoglobin (Hb) and DNA has made significant progress over the past decades, and such adducts have been proposed as biomarkers for use in epidemiological studies aiming to monitor human exposure to toxic agents. In contrast, mathematical models for adduct formation, crucial for the use of these biomarkers for human risk estimation, have been relatively unsophisticated and essentially limited to the linear model. Our work with Hb adduct formation by the neurotoxic agent acrylamide (AA) and its genotoxic metabolite glycidamide (GA) prompted us to develop a nonlinear mathematical model for adduct formation applicable also at the high exposures where capacity-limited processes come into play and toxic effects are commonly observed in experimental organisms.

Humans are exposed to AA mainly in the chemical industry, molecular biology laboratories, and grouting operations (*1*). Acrylamide has for a long time been known to be neurotoxic in animals and man, and more recently its genotoxic and cancer-initiating properties have attracted increasing attention. Despite the fact that AA is carcinogenic in mice when followed by treatment with phorbol esters and is mutagenic in mammalian organisms, it is negative in the Ames test both in the absence and in the presence of S9 mix. Although epidemiological studies have not demonstrated increased rates of tumors in exposed workers, it is classified by the International Agency for Research on Cancer as a possible human carcinogen based on animal bioassays in the mouse and in the rat (*2*).

Because human exposure to AA in most occupational settings is believed to occur through a combination of dermal and inhalational routes (*1*), there is a need for a biomarker of exposure, such as Hb adducts, that is indicative of the total exposure. Initial work on the use of adducts formed by cysteine residues in Hb as biomarkers for AA exposure was performed by Bailey et al. (*3*), who developed a mass fragmentographic technique to determine such adducts in rats given 0–5 mg AA/kg body weight. In this range of concentrations, Hb adduct formation was found to be a convex function of the injected concentration.

This unusual nonlinear relationship between Hb adduct formation and injected concentration, as well as several studies showing an impact of phenobarbital induction on the neurotoxicity of AA in rats, indicated to us that AA underwent further metabolism in a cytochrome P-450-mediated reaction. In an attempt to confirm the hypothesis that AA was metabolically oxidized to the epoxide GA, we analyzed Hb hydrolysates from rats treated with AA and were able to identify *S*-(2-carboxy-2-hydroxyethyl)-cysteine, the adduct formed by GA, by means of GC–MS (*4*) (Fig. 1).

[1]Department of Environmental Health, University of Washington, Seattle, WA 98195.

[2]Department of Applied Mathematics, University of Washington, Seattle, WA 98195.

Address reprint requests to C. J. Calleman, Department of Environmental Health, SC-34, University of Washington, Seattle, WA 98195.

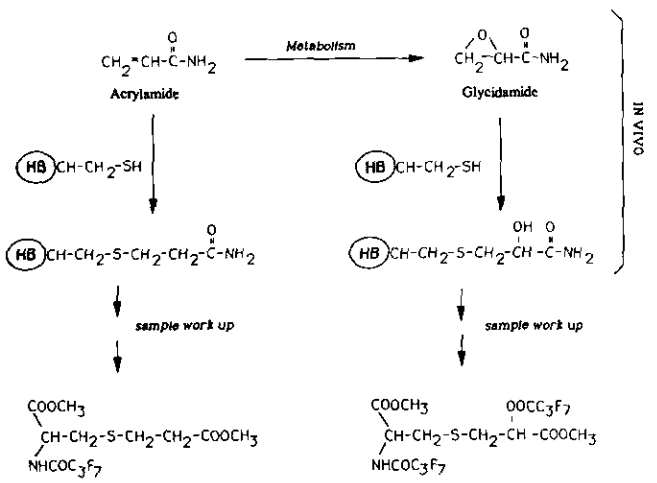

FIGURE 1. Scheme of formation and derivatization of hemoglobin adducts formed by acrylamide and glycidamide.

To evaluate the role of GA in the induction of toxic effects associated with AA exposure, a series of experiments was undertaken to study its toxicological properties and quantitative relevance as a metabolite. It was thus found that AA itself was primarily responsible for causing peripheral neuropathy as judged from the inability of GA to cause neuropathological damage to sciatic nerves or have an effect in the hindlimb splay test in treated animals (*5*, Deng et al., in preparation). On the other hand, GA had a strong impact on body weight, sperm cell viability, and epididymal daily sperm production in male rats (*5*), implicating a role of GA in the induction of male reproductive toxicity in rodents given AA. Already prior to its discovery as a metabolite of AA, GA had been shown to give a positive response in the Ames test (*6*). It was thus hypothesized (*4*) that GA was the agent responsible for the cancer-initiating properties associated with AA exposure in experimental animals.

To be able to measure tissue doses (*7*), D_{AA} and D_{GA}, of both AA and GA, for risk assessment of neurotoxic (D_{AA}) or reproductive and genotoxic effects (D_{GA}), respectively, we developed a GC–MS technique that allows us to simultaneously determine Hb adducts to cysteine formed by both electrophiles (*8*) (Fig. 1). Using this technique, the concentration of adducts formed by GA-(GAHb) in rats was found to be linearly dependent on the injected concentration of GA ($[GA]_0$) and k^*_{GA}, the rate of elimination of GA from the blood, was estimated to 0.48 hr^{-1} from Equations 1 and 2 [cf. Osterman-Golkar et al.(*7*)]

$$D_{GA} = \int_0^\infty [GA]dt = \frac{1}{k_{Hb}} \frac{[GAHB]}{[Hb]} \quad (1)$$

and

$$D_{GA} = \int_0^\infty [GA]dt = \int_0^\infty [GA]_0 \, e^{-k^*_{GA}t} \, dt = \frac{[GA]_0}{k^*_{GA}} \quad (2)$$

Equation 2 is derived from a linear kinetic scenario: $-(d[GA]/dt) = k^*_{GA}\,[GA]$, applicable when $[GA] << K_m$, where rates for saturable processes following Michaelis-Menten kinetics are approximated by V_{max}/K_m and lumped together in the constant k^*_{GA}.

In contrast, adduct formation by either AA or its metabolite GA in rats treated with an initial concentration, $[AA]_0$, of AA in the range 0–100 mg/kg body weight was inconsistent with a linear model. Thus, while adduct formation by AA was convex (*3*), approaching linearity at high concentrations, adduct formation by GA was a concave function of the injected concentration, presumably reflecting a saturable Michaelis-Menten type of kinetics for the metabolic conversion of AA to GA. The following kinetic scenarios for AA and GA, respectively, were thus assumed:

$$\frac{d[AA]}{dt} = k_1[AA] + \frac{V_{max}\,[AA]}{(K_m + [AA])} \quad (3)$$

and

$$\frac{d[GA]}{dt} = -k_2[GA] + \frac{V_{max}\,[AA]}{(K_m + [AA])} \quad (4)$$

in which V_{max} and K_m are the parameters for the metabolic conversion of AA to GA and k_1 and k_2 are the rates of elimination of AA and GA from all other processes. To describe Hb adduct formation by AA and GA in this scenario, the differential Equations 3 and 4 were solved by a transformation of variables and integration of their sum (Calleman et al., in preparation) to yield the expressions:

$$D_{AA} = \frac{1}{k_1}\left\{[AA]_0 - \frac{V_{max}}{k_1} \ln\left(1 + \frac{[AA]_0}{(V_{max}/k_1 + K_m)}\right)\right\} \quad (5)$$

and

$$D_{GA} = \frac{V_{max}}{k_1 k_2} \ln\left(1 + \frac{[AA]_0}{(V_{max}/k_1 + K_m)}\right) \quad (6)$$

The values of the pharmacokinetic parameters giving the best fit to the observed values for Hb adduct formation were 19 μM hr^{-1}, 66 μM, 0.21 hr^{-1}, and 0.48 hr^{-1} for V_{max}, K_m, k_1, and k_2, respectively (Fig. 2). At low concentrations of substrate, where

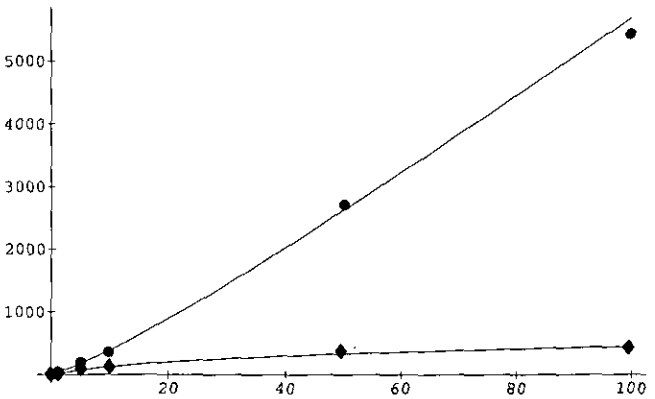

FIGURE 2. Tissue doses of acrylamide (AA) and glycidamide (GA) in rats treated with AA: Equations 5 and 6, with parametrical values inserted for V_{max}, K_m, k_1, and k_2, describing the tissue doses, D_{AA} and D_{GA} (μM/hr), as functions of the initial concentration of AA (mg/kg body weight) in rats. The data points for tissue doses D_{AA} (●) and D_{GA} (♦) were calculated from experimentally determined concentrations of hemoglobin adducts according to Equation 1.

a linear model is applicable, $k^*_{AA} = (k_1 + V_{max}/K_m) = 0.21\ hr^{-1} + (19\ \mu M\ hr^{-1}/66\ \mu M)\ 0.50\ hr^{-1}$ for AA in good agreement with the value 0.40 hr^{-1}, determined by Miller et al. (*9*) from direct measurements of the concentration of AA in the blood of rats. The proportion of AA metabolically converted to GA increases as $[AA]_0$ decreases and approaches $(V_{max}/K_m)/k^*_{AA} = 0.58$ at very low values.

Equations *5* and *6* describe tissue doses and are thus proportional to Hb adduct formation (Equation 1) by a parent compound or a metabolite, respectively, undergoing a saturable process. As capacity-limited processes are commonplace in the metabolism of toxic agents and Equations *5* and *6* are useful also for modeling DNA adduct formation, they merit consideration for incorporation into general models for high-to-low-dose extrapolation of risks associated with exposure to genotoxic/cancer-initiating agents.

In summary, we have developed a method to simultaneously determine Hb adduct formation by AA and its genotoxic metabolite GA in treated rats. Based on the observed nonlinearities in the Hb adduct formation by the two agents, we have developed a novel mathematical model (*10*) that describes adduct formation *in vivo* by agents undergoing saturable metabolic processes and is potentially useful for high-to-low-dose extrapolation of genotoxic effects. By means of this model the values of the pharmacokinetic parameters V_{max}, K_m, k_1, and k_2 were estimated in the rat. Studies are now under way to determine Hb adducts by AA and GA in humans exposed to AA industrially and in molecular biology laboratories as a basis for a quantitative risk estimation of this substance.

This manuscript was presented as a poster at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

This work was financially supported by Cooperative Agreement CR-816768 with the U.S. EPA. Although the research described in this article has been supported by the U.S. Environmental Protection Agency, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.

REFERENCES

1. Office of Toxic Substances. Preliminary Assessment of Health Risks from Exposure to Acrylamide. U.S. Environmental Protection Agency, Washington, DC, 1988.
2. Dearfield, K. L., Abernathy, C. O., Ottley, M. S., Brantner, J. H., and Hayes, P. F. Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. Mutat. Res. 195: 15–77 (1988).
3. Bailey, E., Farmer, P. B., Bird, I., Lamb, J. H., and Peal, J. A. Monitoring exposure to acrylamide by the determination of S-(2-carboxyethyl)cysteine in hydrolyzed hemoglobin by gas chromatography-mass spectrometry. Anal. Biochem. 157: 241–248 (1986).
4. Calleman, C. J., Bergmark, E., and Costa, L. G. Acrylamide is metabolized to glycidamide in the rat: evidence from hemoglobin adduct formation. Chem. Res. Toxicol. 3: 406–412 (1990).
5. Costa, L. G., Deng, H., Gregotti, C., Manzo, L., Faustman, E. F., Bergmark, E., and Calleman, C. J. Comparative studies on the neuro- and reproductive toxicity of acrylamide and its epoxide metabolite glycidamide in the rat. In: Proceedings of the 3rd International Neurotoxicology Association Meeting, Salsomaggiore, Italy, July 1991.
6. Hashimoto, K., and Tanii, H. Mutagenicity of acrylamide and its analogues in *Salmonella typhimurium*. Mutat. Res. 158: 129–133 (1985).
7. Osterman-Golkar, S., Ehrenberg, L., Segerbäck, D., and Hällström, I. Evaluation of genetic risks of alkylating agents. II. Haemoglobin as a dose monitor. Mutat. Res. 34: 1–10 (1976).
8. Beregmark, E., Calleman, C. J., and Costa, L. G. Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. Toxicol. Appl. Pharmacol. 111: 352–353 (1991).
9. Miller, M. J., Carter, D. E., and Sipes, I. G. Pharmacokinetics of acrylamide in Fischer-344 rats. Toxicol. Appl. Pharmacol. 63: 36–44 (1982).
10. Calleman, C. J., Stern, L. G., Bergmark, E., and Costa, L. G. Linear versus nonlinear models for hemoglobin adduct formation by acrylamide and its metabolite glycidamide: implications for risk estimation. Cancer Epidemiol. Biomarkers Prev. 1: 361–368 (1992).